Plasma and red blood cells membrane lipid concentration of sickle cell disease patients

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Objective: The present study aimed to determine the concentration of plasma and red blood cells (RBCs) membrane lipids in Saudi sickle cell disease (SCD) patients.

Methods: This study was carried out at the Hematology Clinic, King Abdul-Aziz University Hospital, Jeddah, Kingdom of Saudi Arabia from October 1998 to October 1999. Lipid concentrations were determined in plasma and RBC membrane of 81 SCD patients and 66 normal healthy matched individuals (control). Different lipid parameters were measured according to standardized enzymatic assay methods.

Results: The plasma concentrations of total cholesterol and low density lipoprotein cholesterol of SCD patients were significantly decreased (p<0.001), whereas the plasma concentrations of high density lipoprotein phospholipids were significantly increased (p<0.001). The plasma concentrations of apo A and apo B were significantly decreased (p<0.001) in SCD patients. However, the concentration of total cholesterol of RBC membrane was significantly increased (p<0.001) in SCD patients, while the phospholipid content was significantly decreased (p<0.001).

Conclusion: The significant increase of RBC membrane cholesterol concentration in SCD patients possibly is responsible to the change in RBC membrane fluidity that may play a direct role in the sickling phenomenon of RBCs in SCD.

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worldwide. The sickle cell gene may be found in association with HbS C, the most common Hb variant in individuals of African origin, producing generally a mild sickling disorder with higher concentration of hemoglobin than HbSS. Also, the coinheritance of α thalassemia (particularly the -α/-α genotype) and β thalassemia with the S gene were found to have marked effects on the red cell indices but little effect on the vaso-occlusive complications of the SCD.

Cardiovascular diseases (CVD) are the major causes of death in adults in many countries and are now considered the most important health problem worldwide. Although several risk factors are reported to be responsible for atherosclerosis, blood lipid levels are closely related to its development. The positive relation of serum cholesterol level to coronary heart disease (CHD) is derived from the low density lipoprotein (LDL) component, whereas high density lipoprotein (HDL) cholesterol is inversely related to risk. The importance of triglycerides as an independent risk factor remains uncertain. The combination of high triglycerides and low HDL-cholesterol often occurs in association with other CHD risk factors such as hypertension and diabetes as is associated with a high risk of CHD. Atherosclerosis can be defined as the development of abnormal fat deposits in the artery wall. The development of atherosclerosis is a complex and multistep process. There are many determinants in the pathogenesis of this condition, with different factors presumably playing key roles at different times in the evolution of the atherosclerotic plaque. A popular theory of atherosclerosis postulates that increased oxidation of LDL predisposes to increased CHD as it enters the arterial wall more readily and is more easily oxidized. The oxidative modification of LDL is believed to play an important role in the development of atherosclerosis. Oxidatively modified LDL has been found in atherosclerotic lesions of humans and experimental animals, and lipid peroxide concentrations have been found to be higher in individuals with atherosclerosis.

The relationship between the effect of sickling and the membrane packing, transmembrane reorientation and distribution of phospholipids in the RBCs, cholesterol levels of cell membrane have been studied. It was shown in SCD red cells that there were a number of changes that include: (i) changes in membrane phospholipid dynamics, (ii) perturbation of the exterior leaflet to the interior leaflet, (iii) perturbation of the interaction between membrane phospholipids and skeletal proteins and abnormal phospholipid molecular species compositions. These changes are believed to be the basis of the vaso-occlusive crisis that is usually seen in many patients with SCD.

Despite the great deal of information available on the hematological and biochemical changes in SCD, indeed very limited studies have been carried out to investigate the lipid profiles in patients with SCD. However, in KSA, there are a very small number of studies on the plasma lipids and lipoproteins in SCD. Moreover, the lipid content of the RBC membrane of Saudi SCD patients as yet, has not been studied. Therefore, the present work was initiated with the aim of determining the plasma and RBCs membrane lipid concentrations in selected SCD patients in order to find out any relationship between SCD and lipid concentrations.

**Methods.** Sickle cell disease Saudi patients (10-25-year-old) from Jeddah, KSA attending the Hematology Clinic at King Abdul-Aziz University Hospital, were recruited for the present study (October 1998 to October 1999). The full hematological parameters were determined, and the clinical manifestations were recorded. The patients were in a steady state and did not receive any blood transfusion for 5-6 weeks prior to sampling. Healthy Saudi individuals of matched age were used as a control group.

**Collection of samples and separation of plasma and red blood cells.** Venous blood cells (10 ml) were collected from subjects, who have been fasting for at least 12 hours, in ethylenediaminetetraacetic acid tubes. The cells were separated from the plasma by centrifugation at 5000g in a refrigerated centrifuge. The plasma was then stored at -80°C until analyzed.

**Determination of different hematological parameters.** Different hematological parameters (number of RBCs, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin) were determined in the blood samples of all the subjects with the use of a hematology analyzer (Celldyne model 3500).

**Extraction of lipids from red blood cells membrane.** The packed RBCs were extracted by Folch solution (Methanol: chloroform, 1:2). The extract was filtered and the filtrate was evaporated to dryness in a rotary evaporator under partial vacuum at 40°C. The residue was then quickly re-dissolved in a known volume of petroleum ether: chloroform (90:10 v/v) and stored at -20°C until analyzed.

**Determination of plasma lipid concentrations.** The plasma concentrations of total cholesterol, triacylglycerol, phospholipids, HDL-cholesterol, and LDL-cholesterol were determined with the use of commercially available diagnostic kits supplied by BioMerieux Laboratory Reagents and Instruments (Marcy, L’Etoile, France) according to standardized assay methods.

**Extraction of lipids and total cholesterol concentrations in red blood cells membrane.** The concentrations of both apo-A1 and apo-B were measured in a Boehringer Mannheim Hitaclin spectrophotometer model 4020. The apo A-1 and Apo B were determined by immunoturbidimetry with the use of monospecific antibodies (supplied by Boehringer Mannheim GmbH, Mannheim, FRG). The concentrations of both apo-A1 and apo-B were determined from a calibration curve established using Apolipoprotein Calibrator.

**Determination of phospholipids and total cholesterol concentrations in red blood cells membrane.** The concentrations of phospholipids and
<table>
<thead>
<tr>
<th>Group (N)</th>
<th>Red blood cells (cells/µL) x 10^6</th>
<th>Hemoglobin (g/dl)</th>
<th>Hematocrit (%)</th>
<th>Mean corpuscular volume (fl)</th>
<th>Mean corpuscular hemoglobin (g/dl)</th>
<th>Serum iron (µmol/L)</th>
<th>Serum ferritin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (66)</td>
<td>5.32 ± 0.05</td>
<td>14.96 ± 0.11</td>
<td>43.29 ± 0.31</td>
<td>81.52 ± 0.48</td>
<td>34.59 ± 0.06</td>
<td>15.29 ± 0.87</td>
<td>201 ± 12</td>
</tr>
<tr>
<td>Sickle cell disease patients</td>
<td>3.36 ± 0.10*</td>
<td>10.32 ± 0.51*</td>
<td>25.26 ± 0.81*</td>
<td>79.51 ± 0.87</td>
<td>33.34 ± 1.23</td>
<td>25.81 ± 1.43*</td>
<td>1050 ± 59*</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM with 'n' as the number of samples. Significant differences between control and other groups were shown: *p<0.001

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control N=66</th>
<th>Sickle cell disease patients N=81</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>3.95 ± 0.13</td>
<td>3.35 ± 0.08‡</td>
</tr>
<tr>
<td>Triacylglycerol (mmol/L)</td>
<td>1.28 ± 0.09</td>
<td>1.29 ± 0.07</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>0.91 ± 0.02</td>
<td>1.04 ± 0.02‡</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>2.27 ± 0.08</td>
<td>1.84 ± 0.05‡</td>
</tr>
<tr>
<td>Total phospholipids (mmol/L)</td>
<td>2.12 ± 0.04</td>
<td>2.31 ± 0.05‡</td>
</tr>
<tr>
<td>Apolipoproteins A</td>
<td>1.33 ± 0.02</td>
<td>1.14 ± 0.03‡</td>
</tr>
<tr>
<td>Apolipoprotein-B</td>
<td>0.98 ± 0.02</td>
<td>0.62 ± 0.02‡</td>
</tr>
<tr>
<td>HDL phospholipids</td>
<td>0.95 ± 0.02</td>
<td>1.08 ± 0.02</td>
</tr>
<tr>
<td>LDL phospholipids</td>
<td>0.95 ± 0.03</td>
<td>0.84 ± 0.03*</td>
</tr>
</tbody>
</table>

HDL - high density lipoprotein, LDL - low density lipoprotein
Results are expressed as mean ± SEM with n as the number of samples. Significant differences between control and other groups are shown *p<0.05, †p<0.01, ‡p<0.001

Results. The results shown in the present investigation were obtained from Saudi patients with SCD and compared with those obtained from normal individuals of matched age. Table 1 shows the values of the hematological parameters of the normal control (n=66) and Saudi patients (n=81) with SCD. The results obtained were typical of SCD in which there was a significant decrease in RBCs count, hemoglobin concentrations, and hematocrit (p<0.001), while serum iron and ferritin concentrations were significantly increased (p<0.001). The plasma concentrations of different lipid classes are presented in Table 2. The plasma concentrations of total cholesterol and LDL-cholesterol of SCD patients were significantly decreased (p<0.001), while the plasma HDL-cholesterol showed a significant increase (p<0.001). Interestingly enough, the ratio of plasma LDL-cholesterol to HDL-cholesterol as well as the ratio of plasma total cholesterol to HDL-cholesterol was significantly decreased (p<0.001) in SCD patients. Furthermore, the plasma concentrations of total phospholipids and HDL-phospholipids showed a significant increase (p<0.001). On the other hand, the plasma concentration of LDL-phospholipids was significantly decreased (p<0.05). The plasma concentrations of both Apo A and Apo B were significantly decreased in SCD patients (p<0.001). The results shown in Table 3 demonstrate a significant increase (p<0.001) in the total cholesterol of RBC membrane of SCD patients. Conversely, the RBC membrane phospholipids content showed a significant decrease (p<0.001). The disturbance in cholesterol and phospholipids of RBC membrane of SCD patients resulted in a significant increase in the ratio of total cholesterol to phospholipids.

Discussion. The RBC is an important source of oxygen-related radicals in SCD. However, sickle RBCs produce greater quantities of \( \text{O}_2^- \), \( \text{H}_2\text{O}_2 \), and hydroxyl radical (OH) than do normal RBCs. Additionally, total cholesterol were determined in the total lipid extract of the RBCs with the use of commercially available diagnostic kits supplied by Bio Merieux Laboratory reagents and instruments as previously described.

Data analysis. The results were expressed as mean ± SEM and comparisons between the 2 sets of data were made by using students t – test. P values <0.005 were considered significant.
sickle RBCs at baseline exhibit increased levels of thiobarbituric acid-reactive substances, suggesting that they are targets for oxidative stress. During deoxygenation of hemoglobin, there is a transfer of electrons between iron atom and O2 leading to the production of O2-. Physiological auto-oxidation of hemoglobin also leads to the production of methemo-globin and O2-. As Hb S auto-oxidizes at 1.7 times the rate of Hb A, SCD patients may have a higher propensity for oxidative production. Within the RBC, one of the targets of oxidant damage is the plasma membrane. In the presence of O2- generating system iron (III) is reduced to iron (II) with subsequent formation of OH and H2O2. The hydroxyl radical oxidizes unsaturated esterified membrane lipids, resulting in changes in fluidity of the bilayer. Cholesterol in RBC membrane normally comprises more than 99% of the neutral lipid in RBC membrane, has been found to exchange freely between both the inner and outer layers of the bilayer and is in equilibrium with non-esterified (free) cholesterol attached to the plasma lipoproteins. However, the rate of exchange between plasma cholesterol remains slow in normal individuals. Therefore, the high ratio of cholesterol to phospholipid in RBC membrane of SCD patients demonstrated in this study due to the increase in the concentration of RBCs membrane cholesterol maybe attributed to the increased rate of exchange between plasma cholesterol and RBC membrane cholesterol and hence, may cause lowering of the concentration of plasma cholesterol by 18% in SCD patients. This proposed increase in the rate of exchange between plasma and RBC membrane cholesterol maybe explained by the loss of RBC membrane phospholipids asymmetry due to an increase in oxidative stress, allowing more plasma cholesterol to be incorporated in the RBC membrane possibly to retain some of the loss in RBC membrane integrity. However, the increase in cholesterol content of RBC membrane of SCD patients by 23% may be responsible, partly, to the change in RBC membrane fluidity that may play a direct role in the sickling phenomenon of RBCs in SCD.

The effect of Hbs and the membrane lipid changes associated with RBC in SCD overrides the protective effect of the observed low plasma concentrations of LDL or cholesterol and the high level of HDL against atherosclerosis. Cholesterol in RBC membrane integrity. However, the increase in cholesterol content of RBC membrane of SCD patients by 23% may be responsible, partly, to the change in RBC membrane fluidity that may play a direct role in the sickling phenomenon of RBCs in SCD.

References